(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78329 A2

(51) International Patent Classification[7]: A61K 38/00

(21) International Application Number: PCT/EP00/05621

(22) International Filing Date: 19 June 2000 (19.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI99A001384 22 June 1999 (22.06.1999) IT

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/78329 A2

(54) Title: THE USE OF THE PROTEIN UK 114 FOR INHIBITING ORGAN TRANSPLANT REJECTION

(57) Abstract: The use of the protein UK 114 for reducing or preventing the rejection of organ transplants, as well as for maintaining transplant acceptance.

THE USE OF THE PROTEIN UK 114 FOR INHIBITING ORGAN TRANSPLANT REJECTION

The present invention relates to the use of the protein UK 114 for reducing or inhibiting the rejection of organ transplants, as well as for maintaining transplant acceptance.

At present the continuous evolution of surgery techniques, together with the development of rather selective, more effective immunosuppressive drugs, have progressively improved the outcome of organ transplants.

However, organ transplantation as permanent solution in case of end-stage organ failure still suffers in practice from the severe problem of the rejection reaction. Treatment with immunosuppressive drugs is used to control such problem, with the consequence that infective complications are still the leading cause of death in transplant recipients. Furthermore, immunosuppressive therapy can never be stopped completely, even after resolution of any acute rejection, but it has to be continued as maintenance treatment indefinitely, although with relatively small doses.

The rejection of allografts (between genetically dissimilar members of the same species) can take place due to either a cell-mediated or a humoral immune reaction against the histocompatibility antigens (HLA) present on the membranes of the donor's cells.

The cell-mediated immune reaction causes graft destruction days to months after transplantation (**acute rejection**) and is characterized by progressive infiltration of mononuclear cells (macrophages, lymphocytes and monocytes) in the transplanted tissue; if these cells perceive antigen differences, they will activate T lymphocytes, which stimulate an immune response, both

cellular (T cells) and humoral (B cells) type, causing the destruction of the transplant. This type of cell-mediated rejection can often be treated with strong immunosuppressive therapy. In case of resolution, a novel acute rejection is unlikely to take place, and the allograft will usually survive for prolonged periods.

The role of humoral antibodies in rejection is clear when the recipient has been presensitized to the HLA antigens present in the graft: in these cases destruction of the transplanted organ takes place within a few hours or even minutes after the revascularization (**hyperacute rejection**).

Chronic rejection is a gradual process of deterioration and failure that occurs later in the life of the transplant, from several months to many years. The immunological mechanisms of the chronic rejection are less clear. The histological picture is different from that of acute rejection, and is characterized by lesions mainly on the arterial endothelium, where extensive proliferation gradually causes occlusion of the vessel lumen, ischemia and fibrosis of the graft.

Immunosuppressive treatment to control organ rejection is at present based on the use of corticosteroids, azathioprine (or cyclophosphamide in case of patients who do not tolerate azathioprine) and cyclosporin, often in a combination thereof.

However, each one of these medicaments involves a number of undesired side effects, which can be summarized as follows:

- corticosteroids: diabetogenicity, increase in proteins catabolism, adrenal cortex atrophy, reduction of the response of connective tissue to lesions, myopathy, osteoporosis, effects on the hematopoietic system and on nervous system);

- azathioprine: depression of bone marrow, hepatitis;
- cyclophosphamide: nefrotoxicity;
- cyclosporin: nefrotoxicity, hepatotoxicity, refractory hypertension and increase in neoplasias.

It is therefore evident the need for an alternative therapy preventing or reducing the rejection of a transplanted organ or tissue, or which anyway increases acceptance by the receiver without involving the above mentioned side effects.

It has now been found, and this is the object of the invention, that the protein having molecular weight of about 14 kDa in SDS-PAGE and obtainable by extraction from mammal liver with perchloric acid, disclosed in WO 97/30154 and in WO 96/02567 and known under the name UK 114, is capable of reducing or preventing rejection in allografts as well as maintaining the acceptance of the graft itself, without inducing the side effects typical of known immunosuppressive drugs.

The protein UK 114 acts on the immune system, exerting pleiotropic effects presumably due to a modulation of the cytokine production by T cells and macrophages.

According to the invention, the protein UK 114 of either extractive or recombinant origin will be administered parenterally, for example through the intramuscular, intravenous, intraperitoneal, subcutaneous, or sublingual routes.

Preferred formulation forms will be injectables forms, such as solutions or suspensions, sterile powders for the preparation of injectable solutions or suspensions; or solid forms such as tablets for the sublingual administration.

The protein of the invention may optionally be administered in combination with other conventional immunosuppressors, such as corticosteroids, azathioprine,

cyclophosphamide, cyclosporin, also in combination thereof.

The dose to be administered will depend on a number of factors, such as the individual characteristics of the patient (weight, etc.) as well as on the type of organ or tissue transplanted or intended for transplantation. In general, however, the amount of UK 114 to be administered will vary from 0.1 to 30 mg/kg/day for a time of 1 to 6 months after transplantation. After that, a maintenance treatment will be followed.

The administration procedures, such as dosage, administration route, duration of the attack and maintenance treatments, possible administration of known immunosuppressive or chemotherapic agents, either concomitantly or separately, will be defined by the skilled clinician.

The following example further illustrates the invention.

Example

Pancreatic islets transplantation in the mouse.

400 to 500 pancreatic islets were prepared from 5-6 week old euglycemic NOD mice, available from Charles River (Calco, Italy), and implanted under the renal capsule of female NOD mice suffering from spontaneous diabetes of recent onset (7-4 days) according to the procedure described by Mellgren A. et al., in Diabetologia, 1986, 29:670. This procedure allows to restore normoglycemia in NOD mice, followed by reappearance of hyperglycemia within 6-8 days, due to the destruction of the transplanted islets (see Sandberg J.O. et al., Clin. Exp. Immunol., 1997, 108:314).

Treatment with UK 114

Starting two days before transplantation, and subsequently every day for the duration of the experiment, three groups of 2-25 week old diabetic NOD mice (15

animals/group) were treated intraperitoneally with UK 114 at a dose of 30 or 60 μg/mouse (in 0.1 or 0.2 ml) or with 0.2 ml of PBS. Glycemia was measured in animals on days 3, 6, 9, 12 and 14 after transplantation from tail blood samples (ExacTech, Baxter Travenol, Deerfield, IL). The animals were considered diabetic when glycemia was higher than 11.8 mmol/l after 6 hour fast.

During the experiment one animal of the control group and another of the group treated with UK 114 at low dosage died on day 2 and 3 after transplantation and they were not considered in the calculation of data.

On day 3 of the experiment, 5/14 mice of the PBS-treated control group were still normoglycemic, on day 6 only one mouse of this group was normoglycemic, and on day 9 all animals were markedly hyperglycemic with glycemia mean values (SD) of 16.2 ± 3.2 mmol/l, which further increased to 18.9 ± 4.5 on day 14 (See table).

Conversely, treatment with UK 114 dose-dependently prevented reappearance of hyperglicemia in transplanted NOD mice. Of the animals treated with 30 μg of UK 114, 12/14 animals were normoglycemic on day 6 and 11 out of 14 on day 9 and 14 (See table). None of the 15 animals treated with the higher dosage of UK 114 became diabetic during the 14 days subsequent transplantation (See table).

These data prove that the administration of UK 114 can prevent, or anyhow delay, rejection of pancreatic islets in a well-known animal model and suggest the potential use of the treatment with UK 114 in the prevention of the rejection of transplanted pancreatic islets in IDDM patients.

TABLE

Days	3	6	9	14
PBS	9/14 diabetic [a]	13/14 diabetic [d]	14/14 diabetic [d]	14/14 diabetic [d]
UK 114 30 µg	2/14 diabetic [b]	3/14 diabetic [c]	3/14 diabetic [e]	3/14 diabetic [e]
UK 114 60 µg	0/15 diabetic [c]	0/15 diabetic [f]	0/15 diabetic [f]	0/15 diabetic [f]

b vs a, $p = 0.02$ with chi-square

c vs a, $p < 0.0001$ with chi-square

e, f, vs d, $p < 0.0001$ with chi-square.

CLAIMS

1. The use of the protein UK 114 for the preparation of medicaments for reducing or inhibiting organ the rejection of transplants and for maintaining the acceptance of the transplant itself.

2. The use as claimed in claim 1, for reducing or preventing the rejection of Langerhans cells transplants.

3. Protein UK 114 as anti-rejection agent.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78329 A3

(51) International Patent Classification[7]: A61K 38/17, A61P 37/06

(21) International Application Number: PCT/EP00/05621

(22) International Filing Date: 19 June 2000 (19.06.2000)

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(26) Publication Language: English

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Published:
— *With international search report.*

(88) Date of publication of the international search report:
19 April 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/78329 A3

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INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/EP 00/05621

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 A61P37/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, MEDLINE, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUSSOLATI G ET AL: "CYTOLYTIC AND TUMOR INHIBITORY ANTIBODIES AGAINST UK114 PROTEIN IN THE SERA OF CANCER PATIENTS" INTERNATIONAL JOURNAL OF ONCOLOGY,GR,EDITORIAL ACADEMY OF THE INTERNATIONAL JOURNAL OF ONCOLOGY,, vol. 10, no. 4, 1 April 1997 (1997-04-01), pages 779-785, XP002040514 ISSN: 1019-6439 page 780, left-hand column, line 29 - line 43 page 784, left-hand column, line 14 - line 19 page 784, right-hand column, line 39 - line 55 page 784, right-hand column --- -/--	3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2000	29/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CECILIANI F ET AL: "THE PRIMARY STRUCTURE OF UK114 TUMOR ANTIGEN" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 393, 16 September 1996 (1996-09-16), pages 147-150, XP002040513 ISSN: 0014-5793 abstract	1-3
P,X	WO 99 43340 A (ZETESIS S.P.A.) 2 September 1999 (1999-09-02) claims 1-4	3
T	PANERAI ALBERTO E ET AL: "Chronic administration of UK-114, a multifunctional emerging protein, modulates the Th1/Th2 cytokine pattern and experimental autoimmune diseases." ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, vol. 876, 22 June 1999 (1999-06-22), pages 229-235, XP000971426 First International Conference on the Neuroendocrine Immune Basis of the Rheumatic Diseases;Genova, Italy; September 18-20, 1998, basis of the rheumatic diseases. June 22, 1999 New York Academy of Sciences 2 East 63rd Street, New York, New York 10021, USA ISBN: 1-57331-215-0 the whole document	1-3

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter al Application No
PCT/EP 00/05621

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9943340 A	02-09-1999	IT MI980356 A	24-08-1999
		AU 2624499 A	15-09-1999